Supporting Information

Butler *et al.* 10.1073/pnas.0807514106

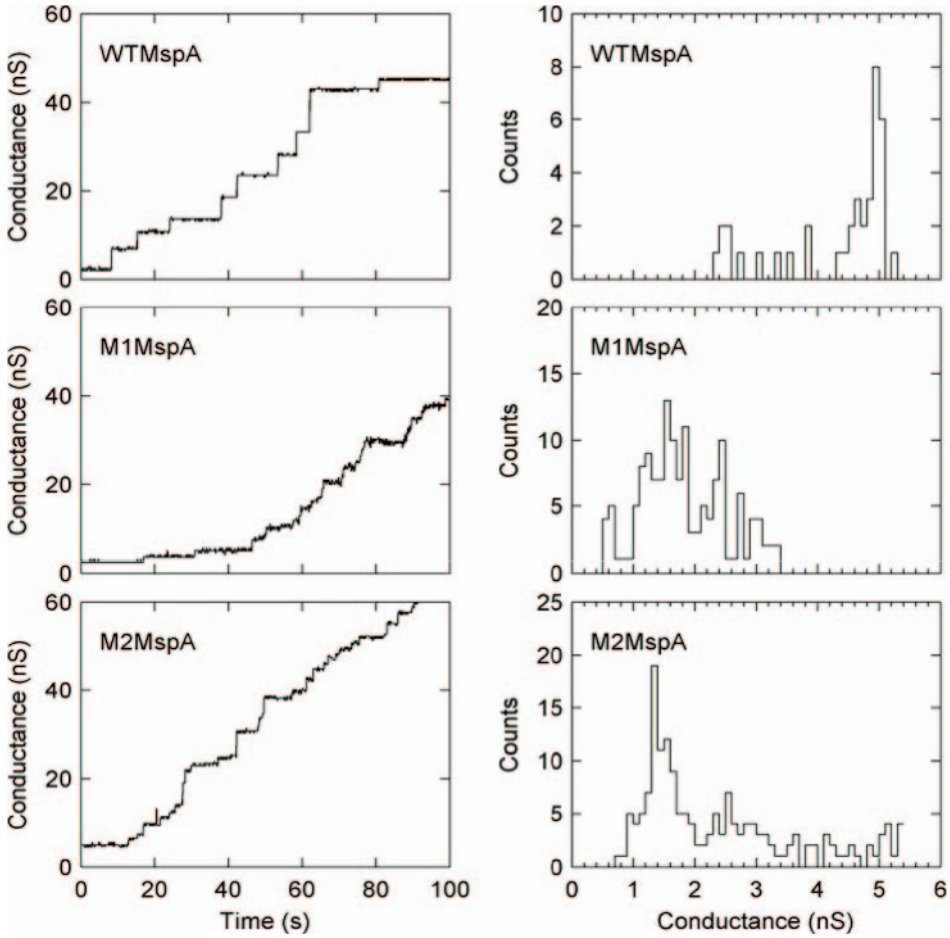

Fig. S1. Assay of channel-forming activity and single-channel conductance for WTMspA, M1MspA, and M2MspA. (*Left*) Shown is bilayer conductance over time when MspA is present in the solution (1 M KCl, 20 °C) bathing the bilayer. Stepwise increases in conductance are interpreted as insertions of MspA pores into the bilayer. (*Right*) Histograms of the sizes of these conductance steps. The WTMspA, M1MspA, and M2MspA histograms summarize 40 insertions from 3 repeated experiments, 144 insertions from 3 repeated experiments, and 169 insertions from 5 repeated experiments, respectively.

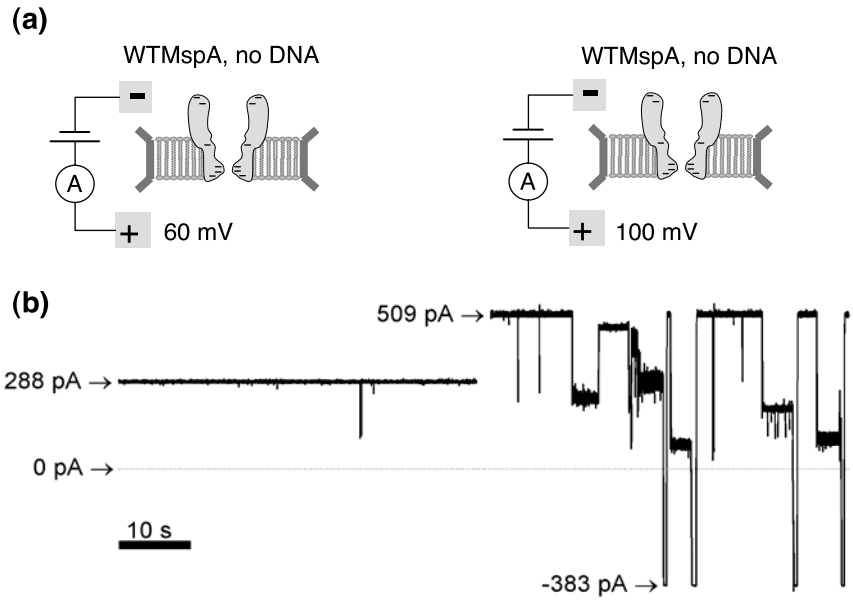

Fig. S2. Spontaneous blockade behavior of WTMspA. (*a*) Schematic diagram of experiments. (*b*) Representative ionic current signals observed for WTMspA at 60 mV (*Left*) and 100 mV (*Right*) with no DNA present. Intervals of negative current flow correspond to reversal of the applied voltage, which was often required to reestablish the unblocked ionic current level.

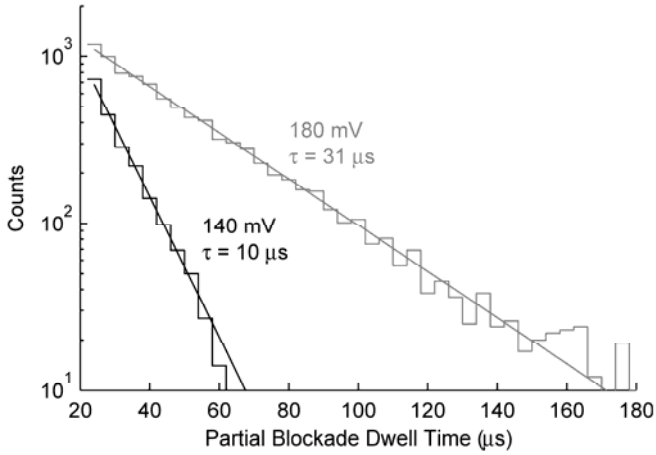

Fig. S3. Partial blockade dwell time distributions for hp08 in M1MspA. Distributions are well-fitted by single exponentials. The partial blockades at 180 mV have a time constant that is a factor of ≈3 longer than at 140 mV.

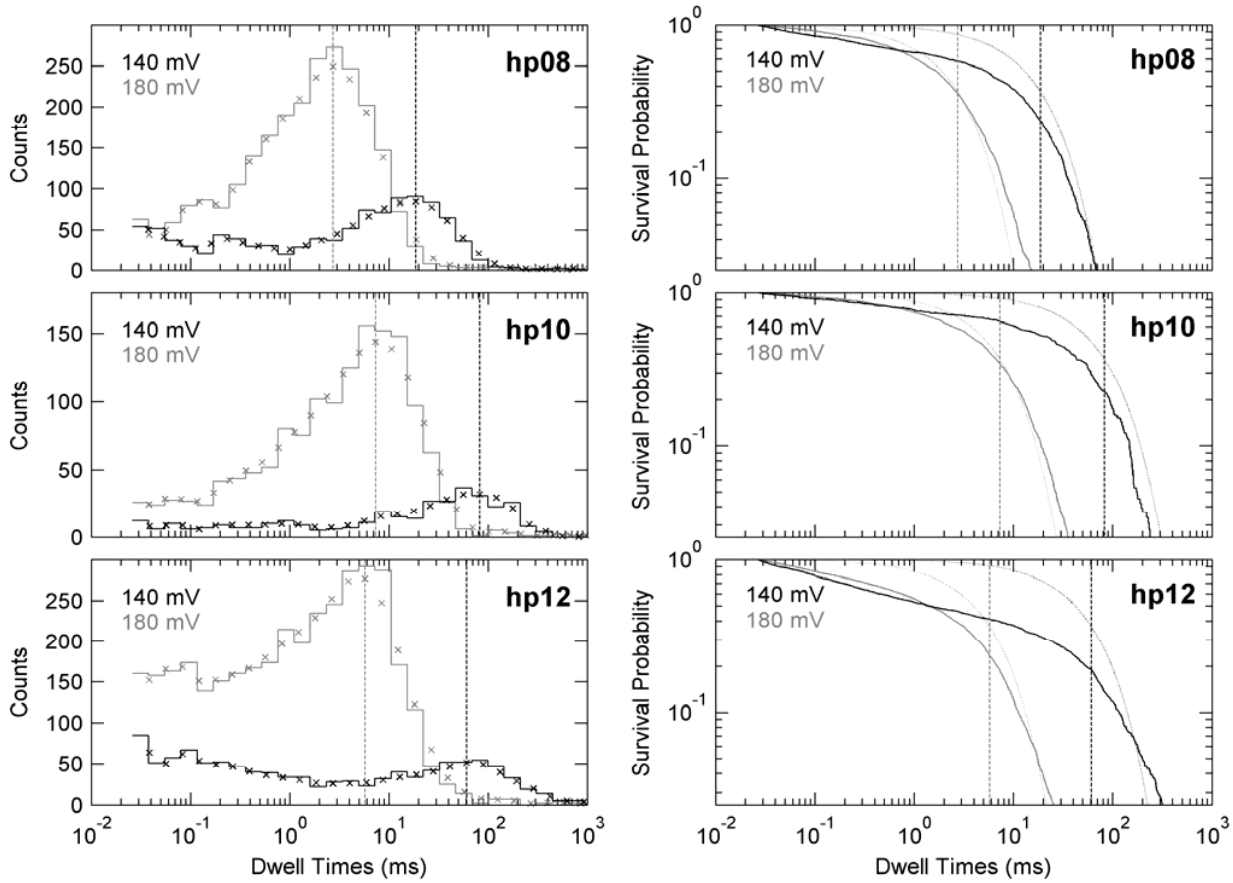

Fig. S4. Detailed look at dwell time distributions of hairpin construct deep blockades in M1MspA. (*Left*) Dwell time histograms with logarithmically spaced bins (stair plots) and corresponding kernel-smoothed density estimates of the probability distribution of the log10 of the dwell times (x). We used the maximum of these smoothed density estimates, t_D, to parameterize, the dwell time distributions. Vertical lines show the t_D values. (*Right*) The survival probability curves derived from the dwell time data (solid lines) and single decaying exponentials, with time constants set to the t_D values of each data set (dashed lines). Our observed data clearly deviate from simple exponential behavior. However, it is reasonable to make qualitative comparison between the t_D value we use and exponential time constants used in other investigations (1) because both parameters reflect similar aspects of the dwell time distributions. Figure derived from same dataset as Figs. 2 and 3.

1. Mathe J, Visram H, Viasnoff V, Rabin Y, Meller A (2004) Nanopore unzipping of individual DNA hairpin molecules. *Biophys J* 87:3205–3212.

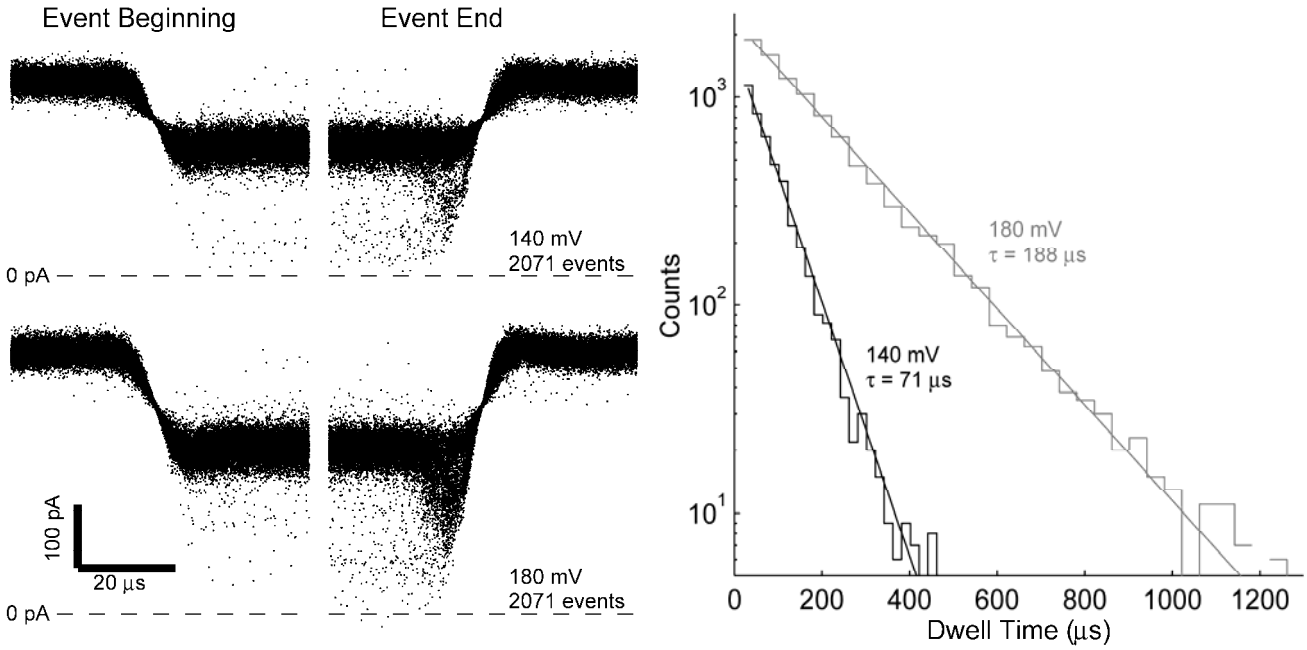

Fig. S5. Statistical characteristics of dT50 blockades in M2MspA. Comparison of the average structure at the beginning and end of the blockades. The figure was created by overlaying the events in a data file aligned at the beginning of the event (*Left*) and at the end of the event (*Right*). Shown is the tendency of blockades to terminate with a brief downward deflection of the ionic current, along with the increase in this tendency with voltage.

Table S1. Strains and plasmids used in this work

Strain/plasmid	Parent strain and relevant genotype	Source
Strain		
E. coli DH5α	*rec*A1, *end*A1, *gyr*A96, *thi*; *rel*A1,*hsd*R17(r_K^-,m_K^+), *sup*E44, ϕ80Δ*lacZ*ΔM15, Δ*lacZ*(YA-argF)UE169	1
M. smegmatis ML16	ML15, ΔmspA::FRT, ΔmspC::FRT, ΔmspD::FRT, attB::loxP, FRT	2
Plasmid		
pMS2	ColE1 origin, PAL5000 origin, Hyg^R	3
pMN016	p_{smyc}-*mspA,* ColE1 origin, PAL5000 origin, Hyg^R	2
pMN035	p_{smyc}-*rv1698,* ColE1 origin, PAL5000 origin, Hyg^R	2
pML904	pMN016 derivative, *mspA* D90N/D91N/D93N (*m1mspA*)	This study
pML840	pML904 derivative, *mspA* D90N/D91N/D93N/D118R	This study
pML841	pML840 derivative, *mspA* D90N/D91N/D93N/D118R/E139R	This study
pML843	pML840 derivative, *mspA* D90N/D91N/D93N/D118R/E139K	This study
pML844	pML843 derivative, *mspA* D90N/D91N/D93N/D118R/E139K/D134R (*m2mspA*)	This study

The annotation Hyg^R indicates resistance to hygromycin. *MspA*, *mspC*, and *mspD* are porin genes of *M. smegmatis*.

1. Hanahan D (1983) Studies on transformation of *Escherichia coli* with plasmids. *J Mol Biol* 166:557–580.
2. Kaps I, *et al.* (2001) Energy transfer between flourescent proteins using a co-expression system in *Mycobacterium smegmatis*. *Gene* 278:115–124.
3. Stephan J, *et al.* (2005) The growth rate of *Mycobacterium smegmatis* depends on sufficient porin-mediated influx of nutrients. *Mol Microbiol* 58:714–730.

Table S2. Oligonucleotides used in this work

Oligonucleotide	Sequence (5′ to 3′ direction)	Purpose
P_{smyc1}	CGACCAGCACGGCATACATC	Amplification and sequencing
pMS-SEQ1	CGTTCTCGGCTCGATGATCC	Amplification and sequencing
MspA909193NFP	CCTGATCAACAACGGTAACATCACCGC	Cloning of pML904
MspA_118R	CTGGGCACGCCTGGGCAACGG	Cloning of pML840
MspA_139R	TCCGGCGCCCGCGGTGGCGTG	Cloning of pML841
MspA_139K	GGCGCCAAGGGTGGCGTGG	Cloning of pML843
MspA_134R	CGTTCTCGGTCCGCGTCTCC	Cloning of pML844

The codons that were altered to introduce the MspA mutations are underlined.